Weed Science, 54:59–68. 2006

Sugars, hormones, and environment affect the dormancy status in underground adventitious buds of leafy spurge (*Euphorbia esula*)

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Signals from both leaves and apical or axillary meristems of leafy spurge are known to inhibit root bud growth. To test the hypothesis that carbohydrates and growth regulators affect root bud growth, decapitated leafy spurge plants were hydroponically treated with glucose, sucrose, gibberellic acid (GA), abscisic acid (ABA), 1-naphthaleneacetic acid (NAA), 6-benzylaminopurine (BA), and a GA biosynthesis inhibitor, paclobutrazol. Both glucose and sucrose caused suppression of root bud growth at concentrations of 30 mM. The inhibitory effect of sucrose was counteracted by GA at 15 μM. In contrast, BA, ABA, NAA, and paclobutrazol inhibited root bud growth at concentrations as low as 1, 2, 1, and 16 μM, respectively. Sugar and starch levels were also determined in root buds at various times after decapitation. Buds of intact plants contained the highest level of sucrose compared with buds harvested 1, 3, and 5 d after decapitation. To determine how seasonal changes affect root bud dormancy, growth from root buds of field-grown plants was monitored for several years. Root buds of field-grown leafy spurge had the highest level of innate dormancy from October to November, which persisted until a prolonged period of freezing occurred in November or early December. Our data support the hypothesis that carbohydrates may be involved in regulating dormancy status in root buds of leafy spurge.

Nomenclature: Leafy spurge, *Euphorbia esula* L. EPHES.

Key words: Carbohydrates, dormancy.

Leafy spurge (*Euphorbia esula* L.) is an invasive perennial weed that is estimated to cause annual economic losses of $130 million in the northern Great Plains (Leitch et al. 1996). Leafy spurge develops root buds less than 2 mo after seed germination, and additional buds form throughout the life of the plant (Coupland et al. 1955). Vegetative propagation through growth of underground adventitious buds on the root and crown, i.e., root and crown buds, is the primary means of reproduction and maintenance of its perennial nature. These buds are maintained in various states of dormancy throughout the seasonal growth cycle but will develop into new shoots if top growth is damaged or separated from the roots under environmental conditions conducive to growth.

Dormancy has been described as the temporary suspension of visible growth of any plant structure containing a meristem (Lang et al. 1987). Dormancy is subdivided into three categories: (1) ecodormancy—growth cessation controlled by external environmental factors, (2) paradormancy (correlative inhibition)—growth cessation controlled by physiological factors external to the affected structure, and (3) endodormancy (innate dormancy)—growth cessation controlled by internal physiological factors. In leafy spurge, paradormancy inhibits buds from developing into new shoots through signals generated from the actively growing aerial portion of the plant. In comparison, endodormancy can be triggered by short-day length and cold temperature (Nooden and Weber 1978).

Various factors including phytohormones, nutrients, water status, and temperature affect root bud dormancy in leafy spurge (Harvey and Nowierski 1988; McIntyre 1972; Nissen and Foley 1987a, 1987b). Since the late 1950s, gibberellic acid (GA) has been known to overcome root bud dormancy in leafy spurge (Shafer and Monson 1958). More recently, Metzger (1994) suggested that sucrose inhibits root bud growth. However, the link between these two substances was not developed until recently. Physiological studies on the responses of root buds to growing apical or axillary-bud meristems and leaves identified two signals, one from mature leaves and one from meristems, causing correlative inhibition (Horvath 1999). The presence of either leaves or growing axillary buds was sufficient to inhibit root bud growth; however, the leaf-derived signal required photosynthesis for its production or transport and could be overcome by the addition of GA. Consequently, it was hypothesized that sugar was the basis for the leaf-derived signal. However, photosynthesis was not required for the signal from growing axillary buds, but auxin transport inhibitors blocked this signal. Current models suggest that the leaf-derived signal is responsible for inhibiting the G1/S-phase transition, and the meristem-derived signal is responsible for inhibition of cell division after the S-phase (Horvath et al. 2002).

Little is known about the signaling mechanisms of leafy spurge that regulate the transition from correlative inhibition to innate dormancy. Some early studies suggested that postsenescence and flowering may induce innate dormancy

(Harvey and Nowierski 1988; Nissen and Foley 1987a). In other plant species, some signals that mediate the induction of innate dormancy have been characterized (see review, Horvath et al. 2003). These signals include light, temperature, ethylene, and abscisic acid (ABA).

The present study was conducted to determine if sugars (sucrose and glucose), growth regulators, and a GA inhibitor promote or inhibit growth of leafy spurge root buds and to determine cellular changes in sugar and starch levels during bud development. Additionally, seasonal effects are known to play important roles in development of innate dormancy and shifts in carbohydrates in leafy spurge crown buds (Anderson et al. 2005). Because root buds of greenhouse-grown leafy spurge are not capable of transitioning into innate dormancy, the growth capacity of leafy spurge root buds was examined using field-grown plants during the years 2000 to 2003.

Materials and Methods

Plant Material

Leafy spurge was started as shoot cuttings using plant material that originated from a wild population in North Dakota (biotype 1984-ND-001) and was maintained by clonal propagation in a greenhouse. Shoot cuttings from greenhouse-grown plants were placed in Sunshine I potting mix[1] inside Ray Leach Cone-tainers[2] and grown in a greenhouse under a 16/8 h day/night photoperiod cycle at 28 ± 4 C for 3 to 4 mo. Plants grown longer than 4 mo often lodge, which would affect auxin transport. Daylight was supplemented with 400-W high-pressure sodium lamps, and light fluencies were approximately 350 μmol m^{-2} s^{-1}.

Plants were left intact (control) or the entire plant above the base of the crown was excised (induced). Crown buds were removed from plants to prevent them from generating signals that would interfere with root bud development. Three replicate plants were used for each treatment, and each experiment was replicated. Root bud growth rate was determined by averaging bud length or by determining the percentage of growing buds. Buds were rated as growing only if they had elongated to at least 3 mm at the time of data collection because dormant root buds usually were less than 3 mm long. Data was collected 8 d after treatment for hydroponic experiments.

Hydroponic System

A hydroponic system was developed to provide plants with various chemical compounds homogeneously. Control and treated plants were put in wide-mouthed, pint-size glass jars with constant aeration through polyethylene tubing 25 cm long and 0.86 mm in diameter (Figure 1). Tubing was connected to an adjustable-screw air valve through a hypodermic needle. All screw air valves were connected to 0.64-cm-diam tygon tubing mounted on a small strip of wood. An aquarium pump was used to provide constant aeration into the solution through the tygon tubing. The jars were covered with aluminum foil, and the lids had a 1.3-cm-diam hole punched in the middle, which was filled with a foam tube plug (size B2). Each foam tube plug was slit to fold around the leafy spurge stem. Also, a 0.86-mm-diam tube passed through the middle hole of the jar for constant aeration. Plants were grown under a 16/8 h day/night photoperiod cycle.

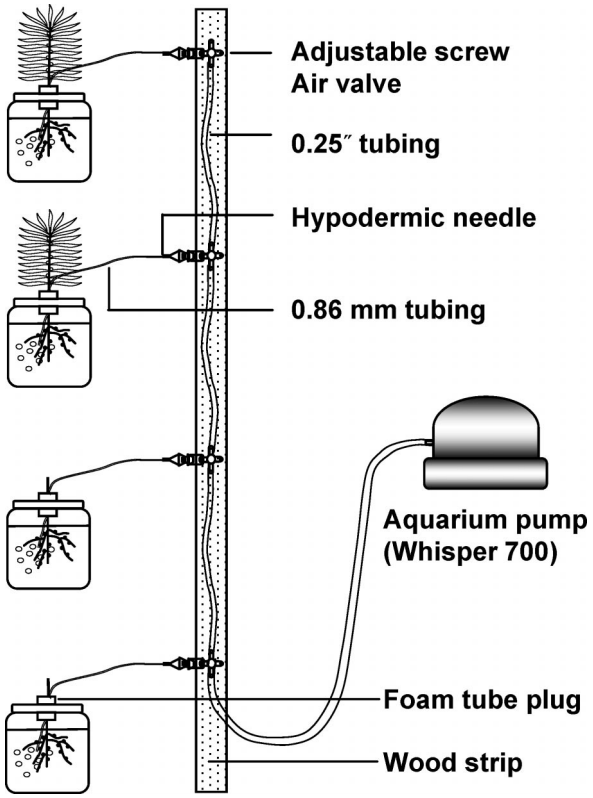

FIGURE 1. Hydroponic system for examining the effects of sugars and growth regulators on developing underground adventitious buds of leafy spurge.

Plant Treatments

Sugar

Our preliminary sugar-response studies indicated that root bud growth was inhibited by 30 mM of glucose or sucrose in Hoagland's solution (data not shown). However, growth in Hoagland's solution presented problems associated with bacteria growth. To solve this problem, sugar dissolved in distilled water, supplemented with 33 μM of potassium nitrate, was used instead of Hoagland's solution. Controls were treated with 33 μM of potassium nitrate only. An osmotic control treatment used 30 mM of polyethylene glycol[3] (PEG) and 33 μM of potassium nitrate solution. Glucose, sucrose, and PEG 400 were used at 30 mM. The pH of the solution was adjusted to 5.7 before use.

Growth Regulators and Chemical Inhibitors

Root bud response to growth regulators and chemical inhibitors was tested hydroponically in 33 μM of potassium nitrate. Growth regulators and chemical concentrations were applied based on published reports (Jacobsen and Olszewski 1993; Pollard and Walker 1990; Soni et al. 1995), and the hormone concentration ranges were also tested experimentally. The concentrations were as follows: ABA,[4] 2 to 4 μM; indole 3-acetic acid[5] (IAA), 100 to 200 μM; 1-naphthaleneacetic acid[6] (NAA), 0.1 to 2.5 μM; GA,[7] 15 to 60 μM; 6-benzylaminopurine[8] (BA), 1 to 40 μM; and paclobutrazol[9]

(Pb), 16 μM. All were directly dissolved in water, except Pb was dissolved in dimethyl sulfoxide (DMSO) and BA was dissolved in 1 N sodium hydroxide (NaOH) before being added into the hydroponic solution. The pH of the solution was adjusted to 5.7 before use.

Seasonal Effects on Dormancy

Innate dormancy was determined by observing the reduction in root bud growth from field-grown plants. Field-grown plants were originally established in 1998 and 1999 using the previously described greenhouse population of maintained leafy spurge (biotype 1984-ND-001). These plants were decapitated (induced) within the first week of each month indicated, and root sections were dug up and grown indoors at 26 C. The buds of field-grown plants were considerably larger in the fall when compared with the greenhouse-grown plants, and many exceeded 3 mm before growth induction (decapitation, see below). Thus, growth rate of root buds for field-grown plants was determined solely by average bud length. Data for field-grown plants were collected 12 d after treatment.

Cellular Sugar and Starch Measurement

Root buds, harvested from intact plants and plants that were grown for 1, 3, and 5 d after decapitation, were immediately transferred to liquid nitrogen (N_2) and stored at −80 C until extracted for analysis of sugar and starch content. Tissue extraction was done following the methods of Gesch et al. (2002). Frozen root buds were ground to a fine powder in liquid N_2. Approximately 250 to 300 mg of frozen crown bud powder was extracted three times in 4 ml of 80% (v/v) ethanol at 85 C. Extracts for each sample were combined and all samples brought to 12 ml. Then, all samples were clarified by adding approximately 200 mg of activated charcoal and by letting stand overnight at 4 C. The clarified solution was removed and evaporated at 60 C overnight, resuspended in 2 ml of deionized H_2O, filtered (0.45 μm),[10] and analyzed for glucose, fructose, and sucrose by high-performance liquid chromatography[11] (HPLC) using a Aminex HPX-87N column[12] and a refractive index detector at a flow rate of 0.5 ml min^{-1} in 0.01 M disodium hydrogen phosphate (Na_2HPO_4). External standards of glucose, fructose, and sucrose were used to standardize the HPLC and were run after every 20 samples as a quality check. The pellet remaining after the hot ethanol extraction was oven dried overnight at 60 C and used for starch analysis. The dried pellet was incubated with 1 ml of 0.2 N potassium hydroxide (KOH) in boiling water for 30 min. After cooling, 0.2 ml of 1 N acetic acid was added, and the solution was incubated with 2 ml of acetate buffer (pH 4.6) containing amyloglucosidase[13] (six units) at 55 C for 1 h. The reaction was terminated in boiling water. After centrifuging at 3,500 × *g* for 1 min, the resulting supernatant was collected and dried at 60 C, resuspended in 2 ml of deionized H_2O, filtered (0.45 μm), and assayed for glucose. Starch measurements are reported as glucose equivalents. The carbohydrate extraction and measurement procedures were performed at least two separate times for each bud sample with two replicate samplings in time.

Histochemistry

Freshly harvested root buds were fixed overnight at 4 C in 4% (wt/v) paraformaldehyde in 50 mM piperazinediethanesulfonic acid (PIPES; pH 6.9) containing 5 mM magnesium sulfate ($MgSO_4$) and 5 mm ethylene glycol-bis(aminoethylether)-tetraacetic acid (EGTA). The samples were then rinsed twice in the same buffer, twice in deionized water, and dehydrated in a graded ethanol series. The last ethanol step included 1% γ-glycidoxypropyl trimethoxysilane,[14] which promoted adhesion of bud scales to the plastic resin (Lindley 1992). After dehydration, the samples were infiltrated and embedded in LR white resin[15] for polymerization at 60 C for 24 h. Samples were sectioned longitudinally at a thickness of 3 μm and affixed to slides coated with 3-aminopropyltriethoxysilane.[16] To detect starch, sections were stained for 5 min with an IKI solution consisting of 1% iodine and 2% potassium iodine in water.

Statistical Analysis

Statistical analysis was done with PC-SAS using the ANOVA procedure. Means were compared with Tukey's multiple comparison procedure or Dunnett's *t* tests at $P = 0.05$ (SAS 1989). Percentage of growing bud data were arcsine-transformed to achieve equal variance (and tests for equal variance substantiate the equal variance assumption); thus, standard errors for these data are the same. Average bud length data were analyzed based on unequal variance.

Results and Discussion

Gibberellic Acid Is Antagonistic to Sucrose in Root Bud Growth

At least two distinct signals cause correlative inhibition of root bud growth (Horvath 1998, 1999). One signal is derived from growing axillary buds and could be blocked by an auxin-transport inhibitor, *N*-1-naphthylphthalamic acid, when applied on the crown section of plants (Horvath 1999). The other signal is derived from mature leaves and requires photosynthesis. To elucidate if sugar is the primary component of the leaf-derived signal, the aerial portion of the plant was excised and roots were hydroponically treated with various concentrations of glucose and sucrose.

Both glucose and sucrose repressed root bud growth at a concentration as low as 30 mM (Figure 2). The inhibition was not due to an increase in osmotic pressure because 30 mM of PEG did not inhibit growth of root buds. The osmotic potential of dilute solutions, with concentrations lower than 100 mM, can be estimated using the van't Hoff equation (Nobel 1999). According to that equation, the solutions tested had an osmotic or water potential of about −0.075 MPa. This is a rather high water potential; inhibition of growth usually requires water potentials of −0.3 MPa or lower. Consequently, it is unlikely that the inhibition of bud growth was attributed to an osmotic effect. The observation that PEG 400 did not inhibit growth supports this notion.

Thus, sucrose or its metabolites could be the leaf-derived signal that inhibits root bud growth. It is known that sugar alone, or through interaction with different phytohormones (ethylene, ABA, GA, or cytokinins), can induce or suppress

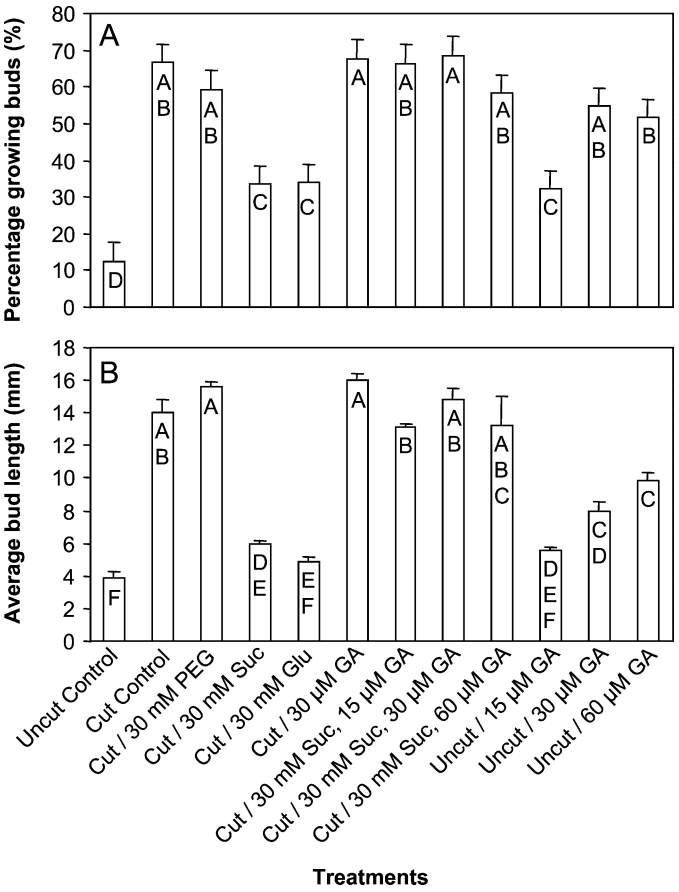

FIGURE 2. Effect of sugar and gibberellic acid (GA) on root bud growth. Intact plants (Uncut control) or decapitated plants (Cut control) served as controls. Several treatments were applied to decapitated plants, including 30 mM polyethylene glycol (Cut/30 mM PEG), 30 mM sucrose (Cut/30 mM Suc), 30 mM glucose (Cut/30 mM Glu), 30 µM GA (Cut/30 µM GA), and 30 mM sucrose, plus various concentrations of GA (Cut/30 mM Suc; 15, 30, or 60 µM GA) for 8 d. Intact plants were also treated with various concentrations of GA (Uncut/15, 30, or 60 µM GA) for 8 d. Data are presented as percentage growing buds (A) and average bud length (B). Vertical bars represent ± SE of the mean, where SE is based on error variance used to compare concentration means. Those means not followed by the same letter are significantly different from each other at P = 0.05 based on Tukey's multiple comparison procedure. Percentage of growing buds data were arcsine-transformed before statistical analysis. The results are combined data of two experiments in time.

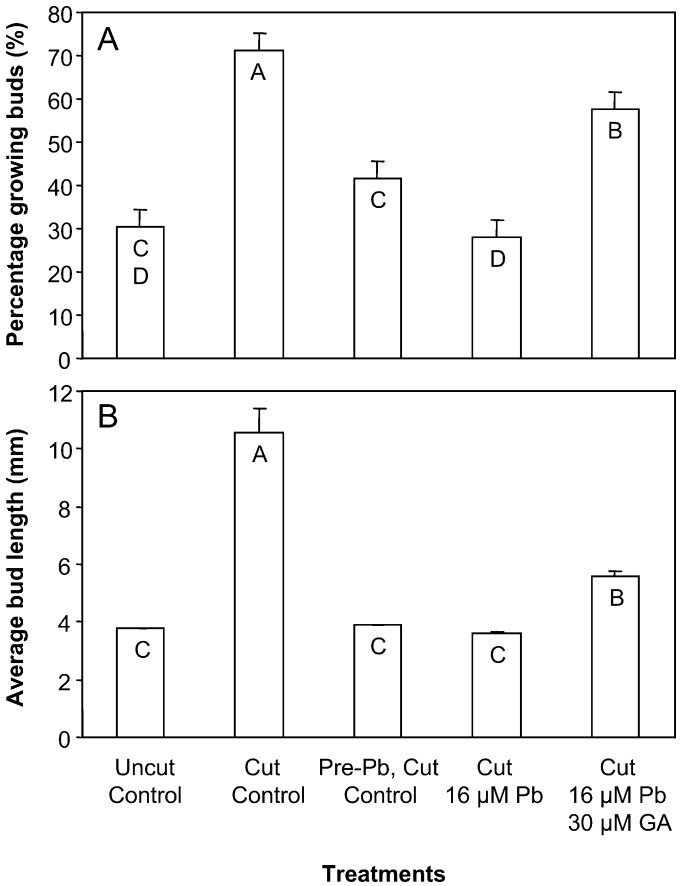

FIGURE 3. Effects of paclobutrazol (Pb) and gibberellic acid (GA) on root bud growth. Intact plants (Uncut control) or decapitated plants (Cut control) served as controls. Intact plants were also pretreated with 16 µM Pb for 2 d, decapitated, and had one of the following treatments performed on them for 8 d: discontinued Pb provision (Pre-Pb, Cut/Control), continually supplied with 16 µM Pb (Cut/16 µM Pb), or supplied with 16 µM Pb and 30 µM GA (Cut/16 µM Pb, 30 µM GA). Data are presented as percentage of growing buds (A) and average bud length (B). Vertical bars represent ± SE of the mean, where SE is based on error variance used to compare concentration means. Those means not followed by the same letter are significantly different from each other at P = 0.05 based on Tukey's multiple comparison procedure. Percentage of growing bud data were arcsine-transformed before statistical analysis. The results are combined data of two experiments in time.

many growth-related genes (Gibson 2004; Smeekens 2000), and hexokinase has been identified as a sensor for sugar (not sugar metabolites) response in higher plants (Jang et al. 1997; Sheen et al. 1999).

GA applied to leafy spurge overcame the inhibition imposed by the leaf-derived signal and induced growth of root buds (Horvath 1999). To determine if GA plays a role in root bud growth after decapitation, the effects of GA and Pb (a GA biosynthesis inhibitor) on growth were examined using the same hydroponic system (Figure 3). Pb inhibited root bud growth at 16 µM (Cut/16 µM Pb); however, intact plants that were pretreated with 16 µM Pb for 2 d, followed by excision of the shoot, and addition of 30 µM of GA showed enhanced root bud growth (Cut/16 µM Pb, 30 µM GA). This result implied that GA was synthesized in the root buds after decapitation, and accumulation of higher GA levels induced root bud growth. Intact plants, pretreated with 16 µM of Pb for 2 d, showed little growth after decapitation and removal of paclobutrazol (Pre-Pb/Cut). This phenomenon would suggest that plants require a recovery time to remove Pb from the system before resuming normal growth.

To determine how GA reacted in the presence of glucose and sucrose, decapitated plants were treated with different concentrations of GA in 30 mM sucrose solution (Figure 2). GA at concentrations as low as 15 µM induced root bud elongation; however, the shoots were thinner than the control. Thus, GA was functionally antagonistic to sucrose, and GA could overcome the inhibitory effect imposed by glucose and sucrose. These results are consistent with the hypothesis proposed by Horvath at al. (2002). Sugar may either exert a negative effect on the synthesis of GA (Perata et al. 1997; Yu et al. 1996) or act as a signal molecule that negatively interacts with the GA signal transduction pathway (Perata et al. 1997).

Although GA appears to be required for root bud growth in leafy spurge, the role of GA in regulating root bud tran-

sition from dormant to active growth is controversial because many studies are correlative in nature. It remains unclear whether GA is directly involved in the dormancy-breaking event or GA synthesis is a product of bud activation. If sucrose is the leaf-derived signal that antagonizes GA action, GA could be the primary compound that breaks quiescence imposed by carbohydrate-induced inhibition. Although GA completely overcame the inhibitory effect of sucrose, it could not induce growth in intact plants to a similar level as that of decapitated plants (Figure 2), indicating that another signal (possibly auxin) is acting separately from the leaf-derived signal.

Effect of ABA, Auxin, and Cytokinin on Root Bud Growth

We have examined root bud growth of leafy spurge in response to several different growth regulators, including ABA, auxin (IAA and NAA), and a cytokinin (6-benzylaminopurine, BA), which are involved in seed and vegetative bud dormancy (Anderson et al. 2001; Foley 2001). It is well known that ABA plays an important role in development of innate dormancy in buds (Horvath et al. 2003), and close relationships between sugar and ABA signal transduction chains have been observed (Finkelstein and Gibson 2001; Smeekens 2000). Abscisic acid also plays a role in imposing and maintaining dormancy in both seeds and vegetative buds (Le Page-Degivry and Garello 1992; Nooden and Weber 1978; Suttle and Hultstrand 1994). Elevated cytokinin levels have been implicated in breaking dormancy in adventitious and axillary buds (Stafstrom 1995). Also, the ratio of cytokinin to auxin controls lateral bud outgrowth (Bangerth 1994; Li et al. 1995). Auxin is likely produced from the expanding meristem and acts indirectly to prevent growth of axillary and adventitious buds (Cline 1991; Leyser 2003). We tested the effects of these growth regulators using our hydroponic system, and then examined the root buds of leafy spurge to see if they exhibited similar responses as those mentioned above.

To determine whether ABA inhibited root bud growth, decapitated plants were hydroponically grown with 2 and 4 μM of ABA (Figure 4). Root bud growth was greatly inhibited by 4 μM ABA but was not inhibited by 2 μM ABA compared with nontreated decapitated plants (Cut control). ABA induces a cyclin-dependant kinase inhibitor (*Ick1*), which reduces the rate of cell division in *Arabidopsis* (Wang et al. 1998). Therefore, the inhibitory effect of ABA on bud growth may be due to reduced cell division in the meristematic region.

IAA at 100 to 200 μM inhibited root bud growth (data not shown). Because the amount of IAA, a natural auxin, used to inhibit root bud growth was high and may be much more than physiological levels, a more stable synthetic auxin, NAA, was also used to determine its effect on bud growth. Root bud growth was greatly inhibited by 1 μM NAA, and at 2.5 μM NAA, the inhibition capacity was similar to that of intact plants (Figure 5). Auxin applied exogenously to leafy spurge root sections reduced root bud growth (Horvath 1998; Nissen and Foley 1987a), whereas a polar auxin transport inhibitor can induce bud growth if leaves are removed (Horvath 1999). Moreover, a *tubulin* gene, which is up-regulated in the late G2-phase of the cell

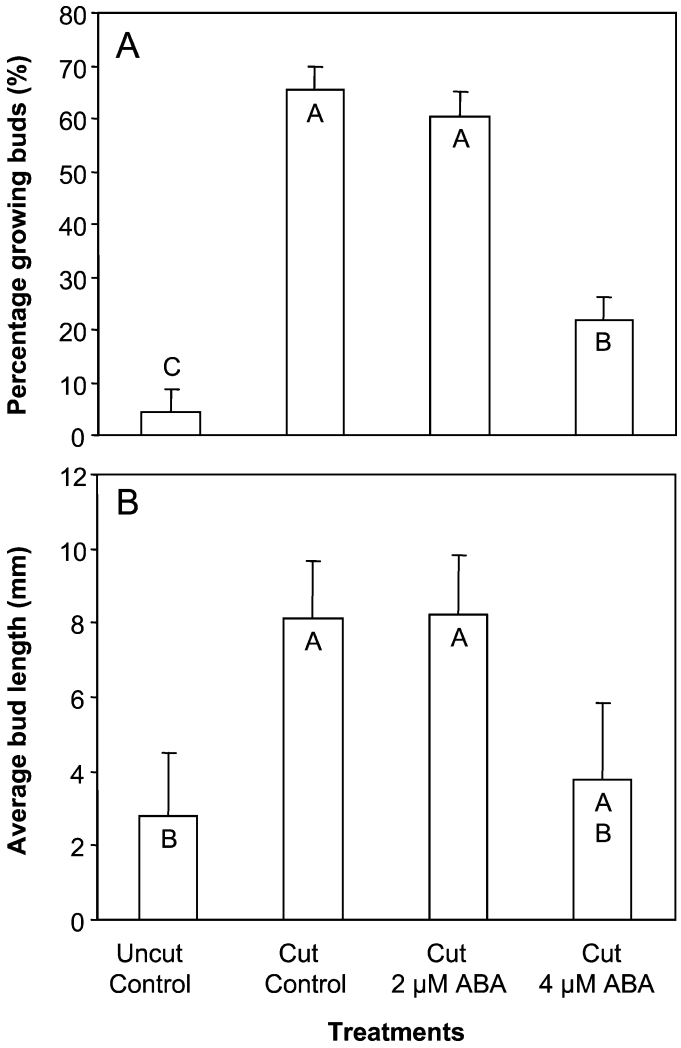

FIGURE 4. Effect of abscisic acid (ABA) on root bud growth. Intact plants (Uncut control) or decapitated plants (Cut control) served as controls. Decapitated plants were also treated with 2 μM (Cut/2 μM ABA) or 4 μM (Cut/4 μM ABA) of ABA for 8 d. Data are presented as percentage of growing buds (A) and average bud length (B). Vertical bars represent ± SE of the mean where SE is based on error variance used to compare concentration means. Those means not followed by the same letter are significantly different from each other at P = 0.05 based on Tukey's multiple comparison procedure. Percentage growing buds data were arcsine-transformed before statistical analysis. The results are combined data of two experiments in time.

cycle, was not expressed unless both the leaf- and meristem-derived signals were removed (Horvath et al. 2002). Thus, auxin may be responsible for inhibition of cell division post-S phase as proposed earlier (Horvath et al. 2002).

Concentrations as low as 1 μM of BA inhibited root bud growth in decapitated plants (compare Cut control with Cut/BA) (Figure 6), and it caused a general swelling of root buds. This result is rather surprising because elevated cytokinin levels have been implicated in breaking dormancy in adventitious and axillary buds (Stafstrom 1995). BA inhibition of root bud growth was not due to an herbicidal effect because visible leaf damage could only be detected with BA at 40 μM in these experiments. To test whether BA action requires the aerial portion of plants, intact plants (Uncut) were treated with various concentrations of BA. BA slightly induced bud growth at a low concentration (compare Uncut

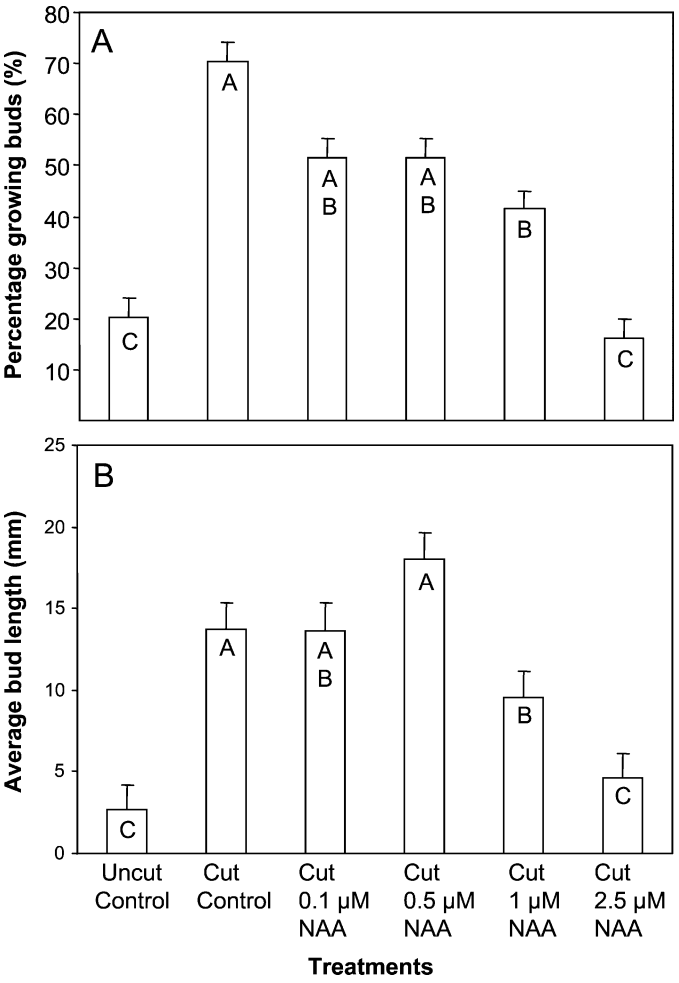

FIGURE 5. Effect of 1-naphthaleneacetic acid (NAA) on root bud growth. Intact plants (Uncut control) or decapitated plants (Cut control) served as controls. Decapitated plants were also treated with 0.1 μM (Cut/0.1 μM NAA), 0.5 μM (Cut/0.5 μM NAA), 1 μM (Cut/1 μM NAA), or 2.5 μM (Cut/2.5 μM NAA) of NAA for 8 d. Data are presented as percentage of growing buds (A) and average bud length (B). Vertical bars represent ± SE of the mean, where SE is based on error variance used to compare concentration means. Those means not followed by the same letter are significantly different from each other at P = 0.05 based on Tukey's multiple comparison procedure. Percentage of growing buds data were arcsine-transformed before statistical analysis. The results are combined data of two experiments in time.

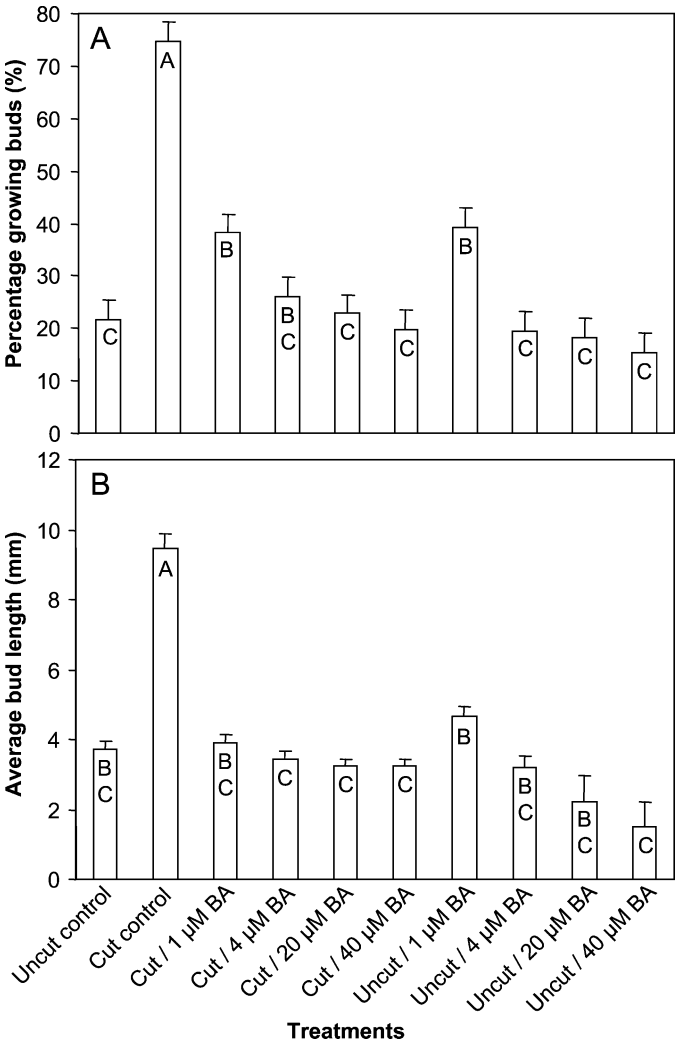

FIGURE 6. Effect of 6-Benzylaminopurine (BA) on root bud growth. Intact plants (Uncut control) or decapitated plants (Cut control) served as controls. Decapitated and intact plants were also treated with 1 μM (Cut/1 μM BA; Uncut/1 μM BA), 4 μM (Cut/4 μM BA; Uncut/4 μM BA), 20 μM (Cut/20 μM BA; Uncut/20 μM BA), or 40 μM (Cut/40 μM BA; Uncut/40 μM BA) of BA for 8 d. Data are presented as percentage of growing buds (A) and average bud length (B). Vertical bars represent ± SE of the mean, where SE is based on error variance used to compare concentration means. Those means not followed by the same letter are significantly different from each other at P = 0.05 based on Tukey's multiple comparison procedure. Percentage of growing buds data were arcsine-transformed before statistical analysis. The results are combined data of two experiments in time.

control with Uncut 1 μM BA) (Figure 6). As BA increased to 4 μM, the growth potential became similar to intact plants. BA of 0.2 μM had no inhibitory effect (data not shown). Thus, among the three growth regulators examined, leafy spurge responded to ABA and auxin in a similar fashion as seeds, axillary buds, and adventitious buds of other plant species (Anderson et al. 2001). However, the response of root buds to BA was unexpected.

Cellular Sugar and Starch Content

Seasonal changes in sucrose and starch have been determined in leafy spurge roots (Cyr and Bewley 1989; Lym and Messersmith 1987) and in crown buds (Anderson et al. 2005). Changes in sucrose and monosaccharide levels during the transition from paradormancy to growth have also been determined in leafy spurge root buds in a controlled greenhouse environment (Horvath et al. 2002). However, the relationship of changes in starch with regard to sucrose, glucose, and fructose has not been previously done in root buds grown under greenhouse conditions.

Changes in cellular sugar content were determined from leafy spurge root buds at various times after decapitation (Figure 7). Root buds of intact plants contained the highest level of sucrose (9.41 ± 0.11 mg g^{-1} fresh weight [fwt]) compared with buds harvested 1, 3, or 5 d after decapitation. Assuming that most of the fresh weight of the buds is water, a sucrose level of 9.41 mg g^{-1} fwt is equivalent to a sucrose concentration of about 28 mM. This value probably underestimates the sucrose concentration in the cytosol and cell sap because components other than water were likely to contribute to the fresh weight of the buds. Thus, sucrose content in root buds of intact plants was similar to, or per-

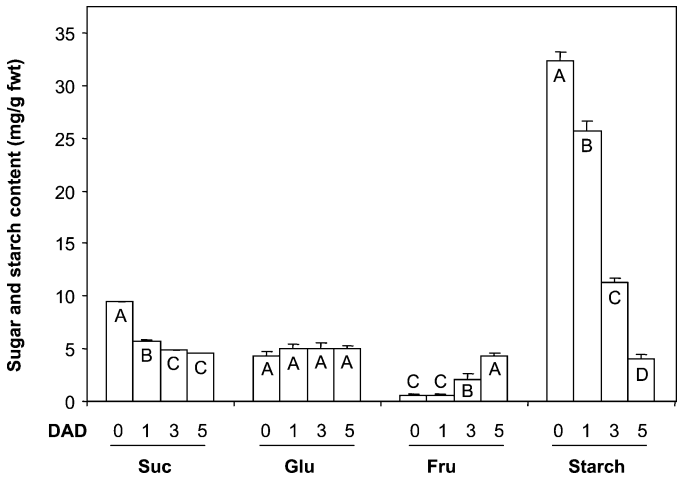

FIGURE 7. Cellular sugar and starch content. Sucrose (Suc), glucose (Glu), fructose (Flu), and starch content (mg g^{-1} fresh weight [fwt]) was determined from leafy spurge underground root buds of intact plants and root buds harvested 1, 3, and 5 d after decapitation (DAD). Error bars represent SE. Those means not followed by the same letter are significantly different from each other at P = 0.05. ANOVA was performed with Proc GLM of SAS. The results are combined data of two experiments in time.

haps even higher than, the 30-mM sucrose concentration used in the hydroponic experiment. Consequently, higher sucrose content in root buds of intact plants correlated well with hydroponic experiment showing that 30 mM sucrose inhibited bud growth (Figure 2).

Sucrose levels decreased significantly (40%) 1 d after decapitation and stayed at similar levels until Day 5. The levels of monosaccharides, glucose (4.26 ± 0.45 mg g^{-1} fwt) and fructose (0.57 ± 0.13 mg g^{-1} fwt), were significantly lower than sucrose in root buds of intact plants. Glucose levels stayed unchanged 1 to 5 d after decapitation, whereas fructose levels increased dramatically during bud growth. A 7.6-fold increase in fructose level was observed at Day 5. A significant increase in monosaccharides was also observed previously at Day 5 after decapitation (Horvath et al. 2002). The present work indicated that the upsurge of monosaccharide levels was because of an increase in fructose. Increases in monosaccharide levels may be indicative of high metabolic activity in the meristematic region.

Changes in cellular starch contents were also determined from root buds at various times after decapitation (Figure 7). Root buds of intact plants contained the highest level of starch (32.4 ± 0.85 mg g^{-1} fwt) when compared with buds harvested 1, 3, or 5 d after decapitation. Starch levels decreased quickly and continuously from Day 1 to Day 5 after decapitation, and at Day 5, the starch level was reduced to 12.5% of the control. Interestingly, a vast decrease in starch had no positive effect on sucrose levels because sucrose content was also reduced (Day 1) or stayed at the reduced level (Days 3 and 5). The observed changes in glucose and fructose are not sufficient to account for the decline in starch. The combined results suggest that the products of starch breakdown are likely rapidly used for cellular respiration or anabolic reactions associated with the early growth of buds.

To further determine where starch granules localized in these buds, freshly harvested root buds were chemically fixed, sectioned, stained with iodine, and observed under a light microscope (Figure 8). A dramatic difference in the amounts of amyloplasts was detected between nongrowing (control) and growing buds (Figures 8a and 8f). Amyloplasts were abundant in control buds, particularly in basal regions of the bud and parenchyma cells of the scales (Figures 8d and 8e). Pith of control buds had conspicuous, but fewer, amyloplast granules (Figures 8b and 8c). In contrast, there were fewer and smaller amyloplasts in buds fixed 3 d after decapitation, and the amyloplasts were virtually absent from the pith region (Figures 8g and 8h). Taken together, the resumption of bud growth appears to be associated with a loss of amyloplastic starch.

Seasonal Effect on Development of Root Buds

Leafy spurge crown buds develop innate dormancy in fall (Anderson et al. 2005; CAB 2004). To determine when root buds develop innate dormancy, field-grown plants were transferred to the greenhouse, decapitated, and allowed to grow indoors (Figure 9). After observing growth potential from whole plants, we found that root buds developed innate dormancy in October and November, after the aerial portion of plants had died down either through senescence or killing frost (See Figure 9; Oct/00 and Oct/01, and Nov/03). However, innate dormancy is quickly lost at the temperature thresholds of November and December. For example, root buds broke innate dormancy in November 2000, December 2001, and December 2003. The samples from 2002 were mishandled, and thus, were excluded in Figure 9.

Photoperiod and temperature may be important factors for imposing physiological changes within underground adventitious buds of leafy spurge. A major shift in starch to sucrose was observed in the crown buds of leafy spurge during the transition to innate dormancy (Anderson et al. 2005). The previous study indicated that sucrose levels started to elevate in September, reached the highest level in December, and began to decline in March, whereas starch levels showed inverse relationships. Seasonal starch and sucrose patterns are possibly alike between root and crown buds because similar sucrose and starch levels were observed in crown and root buds of greenhouse-grown intact plants (data not shown).

Our results showed some inconsistency with the early findings of Nissen and Foley (1987a), in which the capacity for root bud growth was significantly reduced during full flowering. This disparity might be because of differences in research materials; Nissen and Foley used excised root sections whereas we used whole plants. Also, Nissen and Foley took root bud materials from plants of early flowering through late summer (May through August), but fall buds were not examined. The innate-dormancy phenomenon in the fall correlates well with Harvey and Nowierski (1988) observations; they found that at least 42 chilling days were required for leafy spurge to release postsenescence dormancy. In contrast, Nissen and Foley (1987a) used only 8 chilling days to release full flowering–imposed dormancy. Full flowering–imposed dormancy may be caused by the accumulation of relatively high levels of free IAA (Nissen and Foley 1987b) not as a result of shorter day-length and colder temperature at fall.

Conclusions

Interactions between sugars and phytohormones appear to be involved in regulating bud dormancy and growth in

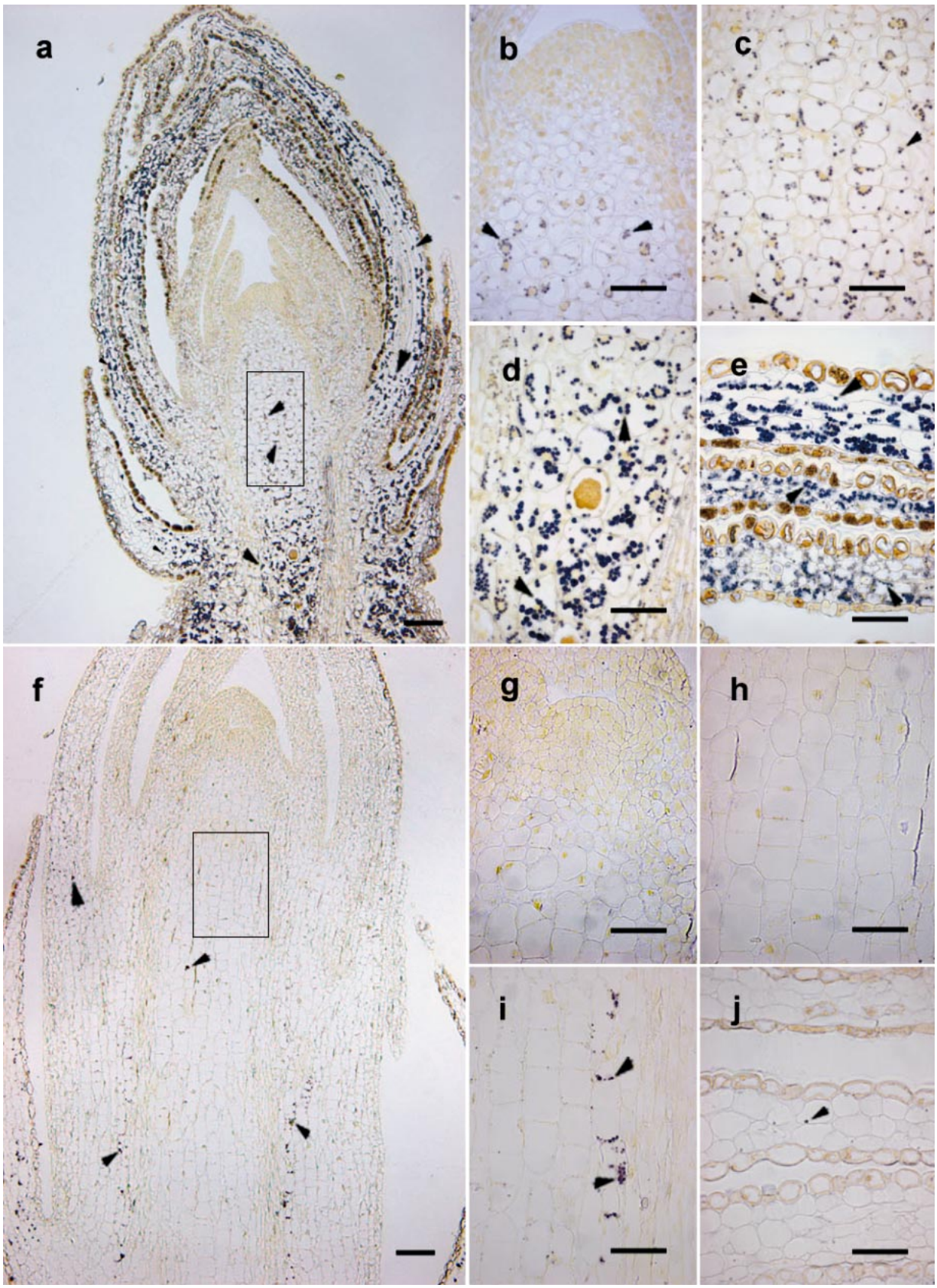

FIGURE 8. Localization of amyloplasts in longitudinal sections of root buds of *Euphorbia esula* after staining with IKI. (a–e) Dormant buds: (a) whole bud; (b) enlarged view of the shoot apical meristem and adjacent cells; (c) enlarged view of the rectangular area marked in (a); (d) enlarged view of basal portion of bud pith; and (e) bud scales. (f–j) Growing buds 3 d after shoot removal: (f) whole bud; (g) enlarged view of the shoot apical meristem and adjacent cells; (h) enlarged view of the rectangular area marked in (f); (i) enlarged view of basal portion of the bud; and (j) bud scales. Arrows point to some amyloplasts. Bars: 100 μm (a and f), 50 μm (b, c, d, e, g, h, i, and j).

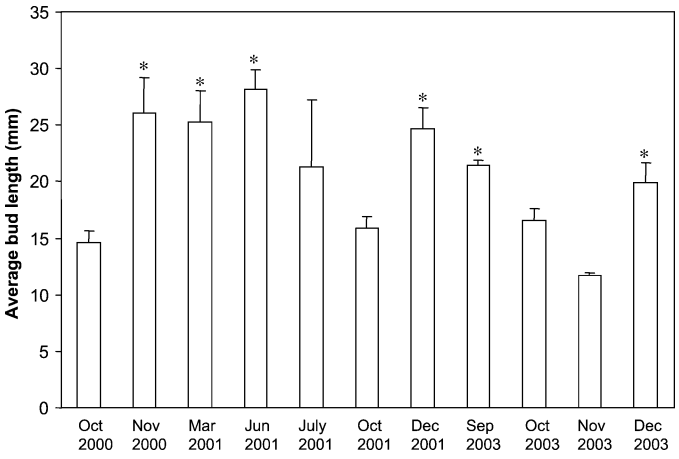

FIGURE 9. Seasonal effect on growth of root buds. Innate dormancy was determined by observing a reduction in root bud growth from the field-grown plants. These plants were decapitated (induced) and grown indoors at 26 C during the years 2000, 2001, and 2003. Bud length data were collected 12 d after decapitation. The data of "Oct 2000" is used as the control in statistical analyses. An asterisk (*) indicates a value significantly higher than the control (Oct 2000) at P = 0.05 as determined by Dunnett's *t* tests. Vertical bars represent ± SE of the mean.

leafy spurge. The results of this study showed that direct application of sucrose and glucose to the roots inhibits root bud growth following decapitation. Furthermore, the concentration of sucrose used in the hydroponic experiments is biologically relevant because a similar concentration was measured in dormant buds. We have also shown that the inhibitory effects of sucrose or glucose are cancelled out by micromolar concentrations of GA. We can only speculate about the nature of the interaction between sucrose and GA. One possibility, however, is that sucrose or its metabolites inhibited the GA response pathway. Alternatively, sucrose may affect the levels of active GAs either by increasing the levels of inactive-conjugated forms of gibberellins or by inhibiting GA synthesis (Gibson 2004). The experiments using Pb support the idea that GA synthesis plays an important role in the resumption of bud growth. Interactions between soluble sugars and GA may also be responsible for regulating starch metabolism in root buds. During bud break, we observed a rapid breakdown of starch. Soluble sugars such as sucrose and glucose have been shown to repress the expression of many α-amylases (Gibson 2004; Koch 1996). In contrast, GA promotes the synthesis and activity of α-amylases in various systems (Jones et al. 1998, Nakayama et al. 2002). Data presented in this article suggest the potential for GA involvement in the breakdown of starch in root buds of leafy spurge during growth induction.

Sources of Materials

[1] Potting mix, Sunshine Mix, Sun Gro Horticulture Inc., 15831 NE 8th Street, Suite 100, Bellevue, WA 98008.

[2] Leach Cone-tainers, SC-10 super cell, Stuewe and Sons Inc., 2290 SE Kiger Island Drive, Corvallis, OR 97333-9425.

[3] P-3640, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[4] A-1049, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[5] I-5418, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[6] N-0640, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[7] G-1025, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[8] B-6750, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[9] 46046, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

[10] 0.45 μm filters, Whatman, Inc. 200 Park Avenue, Suite 210, Florham Park, NJ 07932.

[11] HPLC, Agilent Technologies, 363 Vintage Park Drive, Foster City, CA 94404.

[12] Aminex HPX-87N column, Bio-Rad Laboratories, 1000 Alfred Nobel Drive, Hercules, CA 94547.

[13] Amyloglucosidase, Roche Diagnostic Corp., P.O. Box 50414, 9115 Hague Road, Indianapolis, IN 46250-0414.

[14] γ-glycidoxypropyl trimethoxysilane, SPI Supplies, 569 East Gay Street, P.O. Box 656, West Chester, PA 19381-0656.

[15] LR white resin, London Resin Co. Ltd., P.O. Box 2139, Theale, Berkshire, RG7 4YG, UK.

[16] 3-aminopropyltriethoxysilane, Sigma Chemical Co., P.O. Box 14508, St. Louis, MO 63178.

Acknowledgments

The authors acknowledge Wayne Sargent, USDA-ARS, Fargo, ND, for his technical assistance; Cheryl Kimberlin, USDA-ARS, Fargo, ND, for setting up the hydroponic system and growing leafy spurge; and Drs. Mark West, USDA-ARS, Fort Collins, CO, and Terrance Adams (retired), USDA-ARS, Fargo, ND, for assistance in statistical analysis.

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Received July 5, 2005, and approved September 1, 2005.